

High-Throughput Plating, Treatment, and Lysis Protocols for L1000 Profiling by Cell Type

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Cells: MCF7 (human breast adenocarcinoma cell line; ATCC HTB-22)

Medium: Dulbecco's Modified Eagle's Medium (Mediatech 10013CV) supplemented with 10% v/v fetal bovine serum (Sigma F4135) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:4 when confluent (approximately every 2 days)

Chemical Perturbations

Plating: seed 2,600 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 250 cells in 40µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) and incubate for 24 hours before infection

Cell Infection: add 10µL of 40µg/mL polybrene (Sigma H-9268) per well to a final concentration 8µg/mL, then add 2µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 24 hours then incubate for 72 hours before lysis

Lysis: aspirate 45µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: PC3 (human prostate adenocarcinoma cell line; ATCC CRL-1435)

Medium: RPMI1640 Medium (Mediatech 10040CV) supplemented with 10% v/v fetal bovine serum (Sigma F4135), 1% 100X penicillin-streptomycin-glutamine (Invitrogen10378-016), 1mM sodium pyruvate (Mediatech 25-000-cl, 100mM stock) and 10mM HEPES (Sigma H3537, 1M stock)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:4 when confluent (approximately every 2 days)

Chemical Perturbations

Plating: seed 4,600 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 400 cells in 40µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) and incubate for 24 hours before infection

Cell Infection: add 10µL of 40µg/mL polybrene (Sigma H-9268) per well to a final concentration 8µg/mL, then add 2µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 24 hours then incubate for 72 hours before lysis

Lysis: aspirate 45µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: A549 (human non-small cell lung carcinoma cell line; ATCC CCL-185)

Medium: RPMI1640 Medium (Mediatech 10040CV) supplemented with 10% v/v fetal bovine serum (Sigma F4135) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:6 when confluent (approximately every 2 days)

Chemical Perturbations

Plating: seed 4,000 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 400 cells in 40µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) and incubate for 24 hours before infection

Cell Infection: add 10µL of 20µg/mL polybrene (Sigma H-9268) per well to a final concentration 4µg/mL, then add 1-2µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 3 hours then incubate for 93 hours before lysis

Lysis: aspirate 45µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: A375 (human malignant melanoma cell line; ATCC CRL-1619)

Medium: RPMI1640 Medium (Mediatech 10040CV) supplemented with 10% v/v fetal bovine serum (Sigma F4135) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:6 when confluent (approximately every 2 days)

Chemical Perturbations

Plating: seed 3,500 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 400 cells in 40µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) and incubate for 24 hours before infection

Cell Infection: add 10µL of 40µg/mL polybrene (Sigma H-9268) per well to a final concentration 8µg/mL, then add 1µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 24 hours then incubate for 72 hours before lysis

Lysis: aspirate 45µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: HePG2 (human hepatocellular carcinoma cell line; ATCC HB-8065)

Medium: RPMI1640 Medium (Mediatech 10040CV) supplemented with 10% v/v fetal bovine serum (Sigma F4135) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:4 when confluent (approximately every 2 days)

Chemical Perturbations

Plating: seed 4,000 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 550 cells in 40µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) and incubate for 24 hours before infection

Cell Infection: add 10µL of 40µg/mL polybrene (Sigma H-9268) per well to a final concentration 8µg/mL, then add 1-2µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 24 hours then incubate for 72 hours before lysis

Lysis: aspirate 45µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: VCaP (human metastatic prostate cancer cell line, ATCC CRL-2876)

Medium: Dulbecco's Modified Eagle's Medium (Mediatech 10013CV) supplemented with 10% v/v fetal bovine serum (Sigma F4135) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:3 when confluent (approximately every 6 days)

Chemical Perturbations

Plating: seed 10,000 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 48 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 5,000 cells in 45µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) and incubate for 24 hours before infection

Cell Infection:

KD: replace 25µL media with 25µL of 8µg/mL polybrene (Sigma H-9268) per well at a final concentration 4µg/mL, add 5-6µL per well of virus and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 24 hours then incubate for 24 hours; replace 25µL of media then incubate for 72 hours before lysis

OE: add 10µL of 20µg/mL polybrene (Sigma H-9268) per well at a final concentration 4µg/mL, add 5-6µL per well of virus and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 24 hours then incubate for 72 hours before lysis

Lysis: aspirate 45 μ L medium per well and discard; add 25 μ L TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: HCC515 (human non-small cell lung adenocarcinoma cell line)

Medium: Dulbecco's Modified Eagle's Medium (Mediatech 10013CV) supplemented with 10% v/v fetal bovine serum (Sigma F4135) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:3 when confluent (approximately every 3 days)

Chemical Perturbations

Plating: seed 4,000 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 700 cells in 40µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) and incubate for 24 hours before infection

Cell Infection: add 10µL of 40µg/mL polybrene (Sigma H-9268) per well to a final concentration 8µg/mL, then add 2-3µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 24 hours then incubate for 72 hours before lysis

Lysis: aspirate 45µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: HT29 (human colorectal adenocarcinoma cell line; ATCC HTB-38)

Medium: Dulbecco's Modified Eagle's Medium (Mediatech 10013CV) supplemented with 10% v/v fetal bovine serum (Sigma F4135) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:4 when confluent (approximately every 3 days)

Chemical Perturbations

Plating: seed 4,500 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 900 cells in 40µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) and incubate for 24 hours before infection

Cell Infection: add 10µL of 40µg/mL polybrene (Sigma H-9268) per well to a final concentration 8µg/mL, then add 1µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 24 hours then incubate for 72 hours before lysis

Lysis: aspirate 45µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: HEK293T (human embryonic kidney immortalized cell line)

Medium: Dulbecco's Modified Eagle's Medium (Mediatech 10013CV) supplemented with 10% v/v fetal bovine serum (Sigma F4135) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:6 when confluent (approximately every 2 days)

Chemical Perturbations

Plating: seed 2,600 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: HA1E (human kidney epithelial immortalized cell line)

Medium: MEM-ALPHA (Invitrogen A1049001) supplemented with 10% v/v fetal bovine serum (Sigma F4135) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:4 when confluent (approximately every 2 days)

Chemical Perturbations

Plating: seed 3,500 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) and incubate for 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 400 cells in 40µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) and incubate for 24 hours before infection

Cell Infection: add 10µL of 20µg/mL polybrene (Sigma H-9268) per well to a final concentration 4µg/mL, then add 1-2µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 24 hours then incubate for 72 hours before lysis

Lysis: aspirate 45µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: NPC (human iPS-derived neural progenitor cell line; Stephen Haggarty, PhD, Massachusetts General Hospital)

Medium: Dulbecco's Modified Eagle's Medium (Invitrogen 11995-065) and Ham's F12 (MediaTech 10-080-CV) supplemented with 2% v/v B27 (Gibco 17504-044), 0.1% Human Recombinant Fibroblast Growth Factor-basic (Stemgent 03-0002), 0.1% Human Epidermal Growth Factor (Sigma Aldrich E9644), 0.1% Heparin (Sigma Aldrich H3149) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Maintenance: trypsinize, resuspend, and split 1:3 in the pre-coated flasks when confluent (approximately every 4 days)

Chemical Perturbations

Plating: seed 8,000 cells in 45µL of growth medium per well into 384-well tissue culture-treated plates (Corning 3707) pre-coated with 20µg/mL poly-L-ornithine hydrobromide (Sigma Aldrich P3655) and 5µg/mL laminin (Sigma Aldrich L2020) and incubate for 6 or 24 hours before treatment

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Plating: seed 3,000 cells in 40µL of growth medium per well into 384-well barcoded, tissue culture-treated plates (Corning 8793BC) pre-coated with 20µg/mL poly-L-ornithine hydrobromide (Sigma Aldrich P3655) and 5µg/mL laminin (Sigma Aldrich L2020) and incubate for 24 hours before infection

Cell Infection: add 10µL of 20µg/mL polybrene (Sigma H-9268) per well to a final concentration 4µg/mL, then add 1.5µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace media after 3 hours then incubate for 93 hours before lysis

Lysis: aspirate 45 μ L medium per well and discard; add 25 μ L TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: NEU (neuron cells terminally differentiated in-plate from NPC; Stephen Haggarty, PhD, Massachusetts General Hospital)

Medium: Dulbecco's Modified Eagle's Medium (Invitrogen 11995-065) and Ham's F12 (MediaTech 10-080-CV) supplemented with 2% v/v B27 (Gibco 17504-044) and 1% 100X penicillin-streptomycin-glutamine (Invitrogen 10378-016)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Differentiation & Maintenance: harvest iPS-derived NPC cells with TrypLE (Invitrogen 12563029) between passage 22 and 33 and seed 30,000 cells in 45uL maintenance medium per well into 384-well tissue culture-treated plates (Corning 3707) pre-coated with 20µg/mL poly-L-ornithine hydrobromide (Sigma Aldrich P3655) and 5µg/mL laminin (Sigma Aldrich L2020); replace medium every 2-3 days throughout two-week differentiation

Chemical Perturbations

Treatment:

- a. Differentiated Neurons: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis
- b. Differentiated Neurons stimulated with 100mM KCl: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 2 or 20 hours, then add 5µL of 1M KCl (Applied Biosystems AM9640G) and incubate another 4 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: ASC (primary human adipose-derived adipocytic stem cells; Jeff Gimble, MD PhD, PBRC)

Differentiation Medium: Dulbecco's Modified Eagle's Medium / HAMS F-12 50:50 Mix (Mediatech 15-090-CM) supplemented with 10% fetal bovine serum (Sigma F4135), 1X PSG (Invitrogen 10378016), 0.5mM isobutylmethylxanthine (Sigma I7018), 33 μ M biotin (Supelco 4-7868), 17 μ M D-pantothenate (Sigma P5155), 5 μ M rosiglitazone (Cayman 71740), 1 μ M insulin (Sigma I9278), and 20ng/mL BMP-4 (R&D Systems 314-BP-010)

Maintenance Medium: MesenPRO RS medium (Invitrogen 12746012) supplemented with 10% fetal bovine serum (Sigma F4135), 1X PSG (Invitrogen 10378016), 33 μ M biotin (Supelco 4-7868), 17 μ M D-pantothenate (Sigma P5155), 1 μ M dexamethasone (Sigma D2915), and 1 μ M insulin (Sigma I9278)

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: trypsinize, resuspend, and split 1:3 when confluent (approximately every 3 days)

Differentiation: trypsinize and resuspend pre-adipocytes in maintenance media and seed 5,000 cells in 35 μ L maintenance medium per well into 384-well collagen-coated tissue culture plates (VWR 73521-034), incubate for 48 hours, then replace media with 45 μ L differentiation media for 24 hours before treatment

Chemical Perturbations

Treatment: add 5 μ L of 1:100 diluted 10mM stock compounds per well to a final volume of 50 μ L and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35 μ L medium per well and discard; add 25 μ L TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Cell Infection: add 10 μ L of 20ug/mL polybrene (Sigma H-9268) per well to a final concentration of 4 μ g/mL, then add 6 μ L virus per well and centrifuge for 30 minutes at 2,250rpm in 37°C; replace media after 24 hours with differentiation media; replace media after an additional 24 hours with maintenance media, then incubate 48 hours before lysis

Lysis: aspirate 45 μ L medium per well and discard; add 25 μ L TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: PHH (primary human hepatocyte cells co-cultured with 3T3J2 mouse fibroblasts; David Thomas, MD, Broad Institute)

Medium: Dulbecco's Modified Eagle's Medium High Glucose (Mediatech 15-090-CM) supplemented with 10% fetal bovine serum (Sigma F4135), 1X PSG (Invitrogen 10378016), 21.4 μ M NaHCO₃, 500U/L insulin, 7.5 μ g/mL hydrocortisone, and 7ng/mL glucagon

Incubator: humidified 5% CO₂ atmosphere at 37°C

Maintenance: PHH are plated from frozen vials, 3T3J2 are maintained in Dulbecco's Modified Eagle's Medium without pyruvate (Mediatech 10-027) supplemented with 10% BCS, 1X PSG and split 1:4 when confluent (approximately every 2 days)

Chemical Perturbations

Plating: seed 3,600 PHH cells in 35 μ L of maintenance media per well into 384-well collagen-coated cell culture plates (VWR 73521-034) and incubate overnight; aspirate media and add 6,400 3T3J2 cells in 45 μ L maintenance media per well and incubate briefly before treatment

Treatment: add 5 μ L of 1:100 diluted 10mM stock compounds per well to a final volume of 50 μ L and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35 μ L medium per well and discard; add 25 μ L TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Cells: SKL (human skeletal myocyte cells; Lonza CC-2561)

Differentiation medium: Dulbecco's Modified Eagle's Medium (Invitrogen 11995-065) supplemented with 2% horse serum (Gibco 26050-088) and 1X PSG (Invitrogen 10378016)

Maintenance medium: SkGM media (Thermo NC9465610)

Pre-differentiation maintenance: store in humidified 5% CO₂ atmosphere at 37°C, trypsinize and split population at approximately 1:3 when confluent (every 3-4 days)

Differentiation: trypsinize and resuspend pre-myocytes in maintenance media and seed 5,000 cells in 35µL per well into 384-well collagen-coated tissue culture plates (VWR 73521-034), incubate for 48 hours, then replace media with 35µL differentiation media for 24 hours

Chemical Perturbations

Treatment: add 5µL of 1:100 diluted 10mM stock compounds per well to a final volume of 50µL and incubate for 6 or 24 hours before lysis

Lysis: aspirate 35µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer

Genetic Perturbations

Treatment: add 10µL of 40µg/mL polybrene (Sigma H-9268) per well to a final concentration 8µg/mL, then add 2µL virus per well and centrifuge for 30 minutes at 2,250rpm at 37°C; replace differentiation media and incubate 48 hours; replace media after 48 hours, then incubate 24 hours before lysis

Lysis: aspirate 45µL medium per well and discard; add 25µL TCL Lysis Buffer (Qiagen 1031576) per well; seal plates with Microseal F foil (Bio-Rad MSF-1001); incubate at room temperature for 30 minutes then freeze in -80°C freezer

Storage: -80°C freezer